REMARKS

Claims 1, 6, 12-20, 23-24, 26-27, and 31-35 are pending and claims 1, 31 and 35 have been amended herein. Support for the amendments to claims 1, 31 and 35 can be found in the specification as filed and in originally filed claims 1 and 31. No new matter has been added by these amendments.

Combined Declaration and Power of Attorney

The Examiner noted in Item 4 on page 2 of the Office Action that the oath and declaration filed on March 23, 2001 is defective. Applicants enclose herewith a Supplemental Combined Declaration and Power of Attorney executed by Vimal D. Mehta.

Drawings

The Examiner has indicated that the Draftsman has objected to the drawings. Applicants will file corrected formal drawings upon receipt of a Notice of Allowance.

Claim objections

The Examiner has objected to claims 1, 31 and 35 for including the word "covalent" in brackets. Applicants have herewith amended claims 1, 31 and 35 to remove the term "(covalent)". Thus this objection is moot and can be withdrawn.

Rejection Under 35 USC § 112, second paragraph

Claims 1, 6, 12-20, 23-24, 26-27 and 31-35 are rejected under 35 USC § 112, second paragraph, because claims 1, 31 and 35 recite the term "small molecule," which, according to the Examiner, is not described in the specification. Applicants respectfully call the Examiner's attention to the specification on page 14, lines 13-15, which states "[a]ccording to the invention 'small molecule' may be defined here and in the claims as having a molecular weight of less than 1000D more particularly less than 800D and greater than 50D." Also, the specification at page 6, lines 18-20, indicates that small molecules include dexamethasone and FK506. Thus, Applicants believe that this objection is moot and should be withdrawn.

The Examiner also states that the specification does not give examples or definition for the limitation "pharmacologically relevant small molecules" found in claim 31. Applicants traverse. However, in order to advance prosecution, Applicants have herewith amended claim 31 to remove the phrase "pharmacologically relevant." Thus, this rejection is moot and should be withdrawn.

Further, the Examiner has rejected claims 1 and 35 under 35 USC § 112, second paragraph as being incomplete for omitting essential elements, namely "showing that the bond between the hybrid ligand A and the predetermined target is an irreversible (covalent) bond." (*See* Office Action, page 5). The Examiner also states that "[t]he specification does not show how that the bond is irreversible or how to determine that the bond is irreversible." (*See* Office Action, page 5). Applicants traverse.

Step (a) of claims 1 and 35 as amended herein recites:

"(a) providing a hybrid ligand consisting essentially of two ligands, identified as ligand A and ligand B that are linked together, wherein

(i) ligand A has a specificity for a predetermined target;

(ii) ligand A forms an irreversible bond with the predetermined target;

(iii) and ligand B is the small molecule;"

Part (ii) of step (a) recites that "ligand A forms an irreversible bond with the predetermined target." Thus, Applicants assert that no essential elements have been omitted from these claims.

Moreover, the specification discloses how ligand A forms an irreversible bond with the predetermined target. For example, Figure 3 illustrates the mechanism for aspirin and its analogs for irreversible (covalent) bonding to cyclooxygenase. Further, the specification on page 9, lines 12-16 teaches that aspirin and antibiotics (β-lactams; penicillins and cephalosporins/ cephamycins) have specificity for cyclooxygenase (Cox-1 and Cox-2) and peptidoglycan transpeptidase respectively, to form irreversible (covalent) bond with their targets by acetylation of the amino acid residue, serine hydroxyl group.

The Examiner further states that claims 1, 31 and 35 recite "irreversible (covalent) bond," and that "[t]he specification does not disclose affinities or K_d values for complex formation, to show that the interactions are 'irreversible (covalent bond)." (*See* Office Action, page 5). As

discussed *supra*, claims 1, 31 and 35 have been amended herein to remove the term "(covalent)" from the claims. Additionally, Applicants also call the Examiner's attention to the specification on page 17, lines 15-20, which describe affinities between the ligand or small molecule and the target molecule, states "[t]he affinity of a ligand or small molecule for a target molecule may vary substantially in the chemical-hybrid screen. An example of a range of binding affinities includes a K_d having a value below 10^{-6}, more preferably below 10^{-7} and even more preferably below 10^{-8} and in some embodiments below about 10^{-9}." One of ordinary skill in the art would appreciate that a covalent bond has a dissociation constant on the order of less than 10^{-15}. Thus, contrary to the Examiner's contentions, Applicants believe that no essential elements have been omitted from claim 35.

For all these reasons, Applicants respectfully submit that the rejections to claims 1, 6, 12-20, 23-24, 26-27 and 31-35 under 35 USC § 112, second paragraph have been overcome and can be removed.

Rejections Under 35 USC §§ 102 and 103

The Examiner has rejected claims 1, 6, 12, 17, 19, 20, 23-24, 31-32 and 35 under 35 USC § 102(b) as being anticipated or alternatively, under 35 USC § 103(a) as obvious, over Licitra *et al* ("Licitra") and claims 1, 6, 12, 17, 19, 20, 23- 24, 31 and 35 under 35 USC § 102(e) as being anticipated or alternatively, under 35 USC § 103(a) as obvious, over U.S. Patent No. 5,928,868 to Liu *et al* ("Liu"). Applicants traverse the rejections for the reasons described below.

The Examiner asserts that "[t]he claimed invention does not differentiate or point out how the bond between the ligand A and the target is irreversible, or the formation of the 'irreversible bond' between the ligand A and the target is irreversible, or the formation of the 'irreversible bond' between the ligand A and the target results in a method, which is different from the prior art method." (See Office Action, page 9).

As discussed above, the instant specification discloses both how the bond between the ligand A and the target is irreversible, and how such an 'irreversible bond' is, in fact, irreversible. (See, e.g., page 9, lines 12-16 and Figure 3).

Applicants further assert that the formation of the irreversible bond between the ligand A and the target results in a method which is different from the prior art method of Licitra and Liu, which teach reversible bonds between a ligand and a target.

First, use of the three-hybrid system as disclosed by both Liu and Licitra results in numerous false positive results. (See, *e.g.*, Griffith *et al.*, (2000) Methods ~~in Enzymology~~ 328:89-103 at page ~~101~~, courtesy copy enclosed). For example, in the method of Liu and Licitra any glucocorticoid receptor-interacting proteins present in the expression library will result in transcription of the reporter gene in the absence of binding of the small molecule ligand to its receptor. (See, *e.g.*, Griffith at page 101). In contrast, this result would not be expected to occur using the methods disclosed in the present invention, since, here, ligand A binds irreversibly to its receptor. Moreover, the abundance of false positive results requires the performance of several specificity tests to verify the results. (See, *e.g.*, Griffith at page 101). These specificity tests would not be expected to be required using the methods of the present invention. Thus, the methods of the invention result in greater efficiency by virtue of faster screening and decreased assay costs.

Second, as taught by Licitra, the three-hybrid system cannot be applied to ligands with a K_d value higher than 5nM (See, e.g., Licitra at p. 12820; Griffith at p. 103). This limitation occurs because the three-hybrid system depends on two ligand-receptor interactions as opposed to the single ligand-receptor interaction of the present invention. (See Griffith *et al.*, p. 103). The use of irreversible bonds, including covalent bonds, which typically have K_d values less than 10^{-15}, allows the detection of much lower affinity interactions than with the three-hybrid system as practiced by Liu and Licitra. Thus, a lower concentration of the hybrid ligand can be used in practicing the methods of the present invention.

This ability to use lower concentrations of a hybrid ligand is of particular importance when the ligand (*e.g.*, a drug) is cytotoxic or cytostatic in yeast. Often, drugs introduced into yeast strains cause cell death, or otherwise inhibit yeast transcriptional machinery. Thus, the ability to use lower concentrations of a drug as the hybrid ligand will allow the methods of the present invention to function using drugs that could not be otherwise studied using the three-hybrid system of Liu and Licitra.

Finally, there exists a long felt but unmet need in the art for the improvements to the three-hybrid system that are disclosed in the present invention. In regard to three-hybrid systems, it is generally felt that "[t]he key to the success of these systems is most likely the ligand-receptor pairs, hence one major area of development is new CID (chemical inducers of dimerization) pairs." (*See* Lin and Cornish, (2001) Agnew. Chem. Int. Ed. 40:871-875, at p. 873; courtesy copy enclosed). According to Lin and Cornish, "[o]ne open question is whether other dimerization assays might be even more powerful that the two-hybrid assay-- able to pick up lower affinity interactions . . ." (*See* Lin and Cornish at p. 874).

In summary, Applicants have shown that the disclosures of Liu or Licitra do not render claims 1, 6, 12, 17, 19, 20, 23-24, 31-32 and 35 obvious. Because of the existence of the objective non-obviousness discussed above, Applicants conclude that claims 1, 6, 12, 17, 19, 20, 23-24, 31-32 and 35 are nonobvious over Liu and Licitra. Thus, Applicants respectfully contend that this rejection is improper and should be withdrawn.

CONCLUSION

Applicants submit that the Examiner's rejections have been overcome based on the enclosed amendments and remarks. Applicants therefore respectfully request that the pending claims be found allowable at this time. Should any questions or issues arise concerning the application, the Examiner is encouraged to contact Applicants' undersigned attorney at the telephone number indicated below.

Respectfully submitted,

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VERSION MARKED TO SHOW CHANGES MADE

The Claims:

Claims 1, 31 and 35 have been amended as follows:

1. (Twice amended) A method for identifying a cellular component to which a small molecule is capable of binding, comprising:

(a) providing a hybrid ligand consisting essentially of two ligands, identified as ligand A and ligand B that are linked together, wherein

(i) ligand A has a specificity for a predetermined target;

(ii) ligand A forms an irreversible [(covalent)] bond with the predetermined target;

(iii) and ligand B is the small molecule;

(b) introducing the hybrid ligand into at least one sample, the sample containing an environment, the environment containing;

(i) a first expression vector, comprising DNA encoding the target for ligand A, linked to a coding sequence for a first transcriptional module for expression as a first hybrid protein;

(ii) a second expression vector comprising a random DNA fragment encoding a polypeptide linked to a second transcriptional module for expression as a second hybrid protein; and

(iii) a third vector comprising a reporter gene wherein the expression of the reporter gene is conditioned on the proximity of the first and second hybrid proteins;

(c) permitting the hybrid ligand to bind irreversibly [covalently] the first hybrid protein through ligand A and the second hybrid protein through ligand B so as to activate the expression of the reporter gene;

(d) identifying those samples expressing the reporter gene; and

(e) characterizing the second hybrid protein in the samples identified in (d) so as to determine the cellular component to which the small molecule has a binding affinity.

31. (Twice amended) A kit for detecting interactions between [pharmacologically relevant] small molecules and proteins comprising;

(a) a preactivated ligand A and reagents for forming a hybrid ligand with at least one type of ligand B, wherein ligand A has a specificity for a predetermined target and forms an irreversible [(covalent)] bond with the predetermined target;

(b) a first expression vector comprising DNA encoding a target for ligand A linked to a coding sequence for a first transcriptional module for expression as a first hybrid protein;

(c) a second expression vector comprising a random DNA fragment encoding a polypeptide linked to a coding sequence for a second transcriptional module for expression as a second hybrid protein;

(d) a third vector comprising a reporter gene wherein transcription of the reporter gene is conditioned on the proximity of the first and second hybrid proteins;

(e) an environment for transcription and translation of the first and second hybrid proteins and reporter genes; and

(f) a means for detecting the expression of the reporter gene following the formation of a trimeric complex between the hybrid ligand and the first and second hybrid proteins.

35. (Amended) A method for identifying a cellular component to which a small molecule is capable of binding, comprising:

(a) providing a hybrid ligand consisting essentially of two ligands, identified as ligand A and ligand B that are linked together, wherein

(i) ligand A has a specificity for a predetermined target;

(ii) ligand A forms an irreversible [(covalent)] bond with the predetermined target;

(iii) and ligand B is the small molecule;

(b) introducing the hybrid ligand into at least one sample, the sample containing an environment, the environment containing;

(i) a first expression vector, comprising DNA encoding the target for ligand A, linked to a coding sequence for a first transcriptional module for expression as a first hybrid protein;

(ii) a second expression vector comprising a random DNA fragment encoding a polypeptide linked to a second transcriptional module for expression as a second hybrid protein; and

(iii) a third vector comprising a reporter gene wherein the expression of the reporter gene is conditioned on the proximity of the first and second hybrid proteins;

(c) permitting the hybrid ligand to bind irreversibly [covalently] the first hybrid protein through ligand A and the second hybrid protein through ligand B so as to activate the expression of the reporter gene, thereby reducing a three hybrid system to a two-hybrid system;

(d) identifying those samples expressing the reporter gene; and

(e) characterizing the second hybrid protein in the samples identified in (d) so as to determine the cellular component to which the small molecule has a binding affinity.